

## AN EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF POLYHERBAL FORMULATION (LITHOUT TABLET) BY IN-VITRO MODEL

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### ABSTRACT

*The aim of this study is to prepare & evaluate anti-urolithiatic activity of formulated polyherbal formulation (Lithout tablets) by in-vitro model.*

*The study includes packing of drug and artificial calcium oxalate crystals into the semi permeable egg membrane. After suspending this membrane in the Tris buffer for 7-8 hrs, contents from the membrane collected and added 2 ml of sulphuric acid and titrated with 0.9494 N KMnO<sub>4</sub> solutions.*

*1 ml of 0.9494 N KMnO<sub>4</sub> is equivalent to the 0.1898 mg of Calcium oxalate.*

*We have observed that, according to the titration study weight of the calcium oxalate packed in the semi permeable membrane with test drug was less in quantity as that of the initial weight.*

*Thus, it was concluded that the prepared polyherbal formulation (Lithout tablets) significantly produces anti-urolithiatic activity.*

**KEYWORDS:** Lithout Tablet, Calcium Oxalate, Egg Membrane & Antiurolithiatic

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### INTRODUCTION

Urolithiasis means the formation of stones in the kidney i.e. kidney stone. Urolithiasis is the third most common disorder of the urinary tract, is defined as the formation of sediment in the urinary tract consisting of one or more of the poorly soluble crystalloids of urine. <sup>[1, 2, 3]</sup> It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract stone disease. Among those, kidney stones are common in industrialized nations with an annual incidence of 0.5-1.9%. About 12% of the population of India is expected to have urinary stones <sup>[4]</sup> and out of that about 50% of cases encounter loss of one or both kidneys with or without renal damage up to some extent. Nearly 15% of the population of northern India is also suffering from kidney stones. Upper as well as lower urinary tract stones occur frequently but the incidence shows wide variation on the regional basis in India. <sup>[5]</sup> Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly costly and with these procedures recurrence is quite common. <sup>[1, 2, 6]</sup> Several allopathic drugs also there in the market but has side effects thus most of the people turns towards the herbal formulations. We have prepared a polyherbal formulation (Lithout tablets)

containing seven different herbal drugs which have anti-urolithiatic activity mentioned in the Ayurveda. In this study we have used the titrimetry model to prove the activity of the formulation.

## **MATERIALS AND METHODS**

### **Plant Material**

Powders of herbal drugs were collected from the local market of Pune City.

### **Methods**

#### **Preparation of Tablets**

Tablets of powdered herbal drugs were prepared by the wet granulation method and evaluated as per the Indian Pharmacopoeia.

#### **Preparation of Experimental Kidney Stones (Calcium Oxalate) by Homogeneous Precipitation**

Equimolar solution of calcium chloride dihydrate (AR) in distilled water and sodium oxalate (AR) in 10 ml of 2 N  $\text{H}_2\text{SO}_4$  was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. The precipitate freed from traces of sulphuric acid by ammonia solution. Washed with distilled water and dried at  $60^\circ\text{C}$  for 4 hrs.

#### **Preparation of Semi-Permeable Membrane from Farm Eggs**

This membrane lies in between the outer calcified shell and the inner contents like albumin and yolk. The shell was removed chemically by placing the eggs in 2M HCl for 24 hrs, which caused complete decalcification. Further, washed with distilled water and carefully with a sharp pointer, a hole is made on the top and the contents squeezed out completely from the decalcified egg. It was washed thoroughly with distilled water, and placed in ammonia solution, in the moistened condition for a while and then rinsed with distilled water. Finally it is stored in refrigerator at a pH of 7-7.4.

#### **Method for Evaluation of Anti-Urolithiatic Activity by Kramer and Tisdall Method**

In-vitro antiurolithiatic activity can be studied by inducing calcium oxalate stone formation and studying the effect of plant extracts on their ability to decrease the amount of calcium oxalate. In the present study Kramer and Tisdall method was used to evaluate In-vitro antiurolithiatic activity.

After preparing semi-permeable membranes exactly 1 mg of calcium oxalate and 10 mg of various drugs/ compounds/ standard was weighed and packed in semi-permeable membrane separately and carefully sutured. This was allowed to suspend in a conical flask containing 100 ml of 0.1M TRIS buffer. All the conical flasks were maintained at room temperature undisturbed for 7-8 hours. The remaining contents in the semi-permeable membrane were transferred into a test tube. 2 ml of 1N sulphuric acid was added and titrated with  $\text{KMnO}_4$  till colour change was obtained.

#### **Equivalent Factor**

1 ml of 0.9494 N  $\text{KMnO}_4$  equivalent to 0.1898 mg of Calcium.

The amount of undissolved calcium oxalate is then subtracted from the total quantity used in the experiment in the beginning. <sup>[8, 9]</sup>

## RESULTS

Table 1: Calcium Oxalate Dissolution by Test and Standard Drug

Group	Volume of Standard KMnO <sub>4</sub>	Wt. of Calcium Estimated	Wt. of Calcium Reduced	% Dissolved
Control	0.001 ml	0.001898 mg	--	--
Standard (Cystone)	0.15 ml	0.02847 mg	9.9715 mg	93.33%
Test Drug	0.1 ml	0.01898 mg	9.9810 mg	90.00%

\*Corresponds to 10 mg

## DISCUSSIONS AND CONCLUSIONS

In this study, we prepare a polyherbal formulation consist of seven various potent antiurolithiatic herbs (Lithout tablets) and the anti-urolithiatic activity of Lithout tablet was evaluated by using titrimetry model. In titrimetry model potassium permanganate, calcium oxalates were used. Potassium permanganate is a reducing agent which reduces the amount of calcium oxalate. So this is useful to estimate the remaining amount of calcium oxalate in the egg membrane. The egg membrane was prepared by using raw eggs and 1:1 HCl. About 80% of kidney stones are made up of calcium oxalate crystals. The study reveals that weight of calcium oxalate was reduced in comparison with initial amount of calcium oxalate. Further, we observed that the % of calcium oxalate dissolution capacity. The % calcium oxalate dissolved was 93.33 % and 90 % in Standard & Lithout Tablet respectively (See Table 1). The efficiency of dissolution of oxalate crystals of Lithout is slightly less as compared with the standard drug (Cystone) but it produces significant anti-urolithiatic action. This in-vitro study gives significant data that proves the antiurolithiatic activity of the Lithout tablet.

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